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South African Pilchard Oil

7. THE ISOLATION AND STRUCTURE OF AN OCTADECATETRAENOIC ACID FROM SOUTH AFRICAN PILCHARD OIL*

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Toyama & Tsuchiya (1935) isolated an octadecatetraenoic acid (moroctic acid) from sardine oil and assigned to it a 4:8:12:15-tetraene structure. Paschke & Wheeler (1954) reported the presence of a tetraethenoid octadecanoic acid in the freshwater alga *Chlorella pyrenoidosa*, but were unable to isolate it. By analogy with other acids isolated from this source, and from spectroscopic evidence, they considered a 6:9:12:15-tetraene structure as most probable. Recently, the isolation of octadeca-6:9:12:15-tetraenoic acid from herring oil was reported by Klenk & Brockerhoff (1957). The evidence for the presence of such an acid in pilchard oil was also obtained in this Laboratory (see Sutton, 1957, quoting unpublished work by Whitcutt).

The isolation of an octadecatetraenoic acid from South African pilchard oil (*Sardina ocellata* Jenyns) is now reported. The methods of separation and identification were the same as those described in previous papers of this series (see Silk & Hahn, 1954*b*; Whitcutt & Sutton, 1956). The acid is shown to be the all-*cis*-*n*-octadeca-6:9:12:15-tetraenoic.

EXPERIMENTAL AND RESULTS

Melting points are uncorrected. General methods were described in previous papers of this series.

Infrared and ultraviolet spectra were determined on Perkin–Elmer model 21 and Unicam SP. 500 spectrophotometers respectively.

* Part 6: Whitcutt, 1957

Isolation of the octadecatetraenoic acid

Molecular distillation of the unsaturated esters. The starting material for the isolation of the octadecatetraenoic acid was distillate 1, Table 1 of part 6 of this series (Whitcutt, 1957), which was prepared by lithium salt–acetone and urea-complex procedures for segregation of the acids,

Table 1. *Molecular distillation of distillate* 1, *Table* 1, *part* 6 *of this series* (*Whitcutt*, 1957)

Fraction no.	Distillation temp.	Wt. (g.)
1	60–70°	39·9
2	70	9·7
3	70	7·5
4	70	5·9
R (Residue)	—	69·0

Table 2. *Molecular distillation of fraction* 1, *Table* 1

Fraction no.	Distillation temp.	Wt. (g.)
1*a*	52°	1·85
1*b*	56	3·35
1*c*	56	6·20
1*d*	60	4·94
1*e*	60	3·71
1*f*	60	3·05
1R (Residue)	—	13·20

Table 3. *Molecular distillation of fraction* 1*R*, *Table* 2

Fraction no.	Distillation temp.	Wt. (g.)
1RD	64°	6·25
1RR (Residue)	—	2·93

and molecular distillation of the esters. Distillate 1 (134·8 g.) was subjected to a further series of molecular distillations in the still described previously (Sutton, 1953). Fractions shown in Tables 1–3 were obtained. Fraction 1 RD (Table 3) was saponified and treated in the manner described previously (Whitcutt, 1957) to give a concentrate of highly unsaturated acids (Found: equiv. wt., 271; iodine value, 378). A part of it (10 mg.) was hydrogenated over Pd–$BaSO_4$ catalyst and the hydrogenation product subjected to chain-length analysis (Silk & Hahn, 1954*a*). It consisted of palmitic, stearic and arachidic acids, representing about 50, 36 and 14% respectively of the total.

Reversed-phase partition chromatography of the concentrate 1 RD. When the concentrate (10·7 mg.) obtained as described above was chromatographed on a small (0·9 cm. × 100 cm.) hydrophobic kieselguhr–heptane column (Howard & Martin, 1950) clear separation into four components was obtained (Fig. 1). Unfortunately, the separation was far less complete on a preparative scale (500 mg. of material; 4 cm. × 125 cm. column), as shown in Fig. 2. Fractions 1–4 (Fig. 2) were collected and treated as described previously. Altogether, about 300 mg. of fraction 3, representing octadecatetraenoic acid, was recovered from seven chromatograms, but corresponding samples were not mixed.

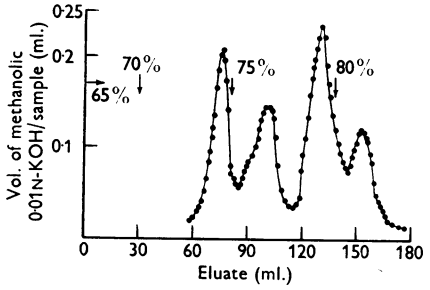

Fig. 1. Chromatogram of fraction 1 RD (Table 3). Weight of the sample, 10·7 mg. Column, heptane supported on non-wetting kieselguhr, 0·9 cm. × 100 cm. Temperature, 10°. Flow rate, 45 ml./hr. Arrows indicate changes in the concentration of eluting solvent (aqueous methanol; %, v/v).

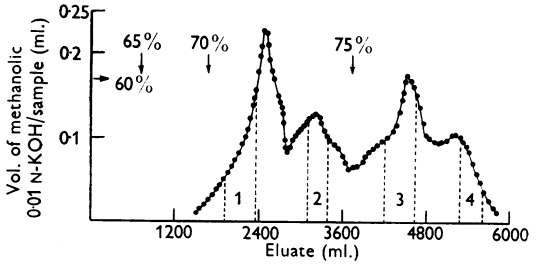

Fig. 2. Preparative chromatogram of fraction 1 RD (Table 3). Weight of the sample, 0·5038 g. Column, heptane supported on non-wetting kieselguhr, 4 cm. × 125 cm. Temperature, 10°. Flow rate, 300 ml./hr. Arrows indicate changes in the concentration of eluting solvent (aqueous methanol; %, v/v). Collected fractions are shown by broken lines.

Fractions 1 and 4 of Fig. 2 were found to consist of the hexadecatetraenoic and eicosapentaenoic acids respectively, which have previously been described in this series (Silk & Hahn, 1954*b*; Whitcutt & Sutton, 1956).

Chain-length analysis of fraction 2 (Fig. 2) indicated that it was a mixture of C_{16} (about 70%) and C_{18} (about 30%) acids. It had equiv. wt. 259, absorbed 3·68 moles of hydrogen/equiv. over Pd–$BaSO_4$, and apparently contained an unidentified hexadecatrienoic acid. This fraction was not investigated further.

Properties and structure of the octadecatetraenoic acid. The octadecatetraenoic acid recovered from fraction 3, Fig. 2, was a pale-yellow oil of m.p. −57·4 to −56·6°, refractive index, n_D^{16} 1·4888 [Found: C, 78·2, H, 10·5%. Number of double bonds/mol., 3·9 (hydrogen uptake over Pd–$BaSO_4$); equiv. wt., 277; iodine value, 343. $C_{18}H_{28}O_2$ requires C, 78·2, H, 10·2%; number of double bonds/mol., 4·0; equiv. wt., 276; iodine value, 367].

Except for a slight inflexion at 235 mμ ($E^{1\%}_{1\,cm.}$ 3·3) corresponding to 0·36% autoxidation (Silk & Hahn, 1954*b*) the acid showed no absorption in the 210–300 mμ region in ultraviolet light (see Fig. 3).

The infrared spectrum (Fig. 4) was taken in CS_2 solution (1·57%, w/w) except from 1650 to 1320 cm.$^{-1}$, where CCl_4 solution (1·44%, w/w) was used.

Hydrogenation product. Chain-length analysis of the hydrogenation product showed the presence of stearic acid, together with about 4% of palmitic acid and smaller amounts of arachidic acid.

The unsaturated acid (66 mg.) was hydrogenated over Pd–$BaSO_4$ in acetic acid and treated in the usual way. After two crystallizations from hexane at 4°, the saturated acid obtained had m.p. 67·6–68·2°, which was raised on admixture with authentic stearic acid of m.p. 69·0–69·4° to 68·4–69·0° (Found: C, 76·1, H, 13·0%; equiv. wt., 279. $C_{18}H_{36}O_2$ requires C, 76·0, H, 12·8%; equiv. wt., 284).

The X-ray long spacing of the product was 39·99 Å, compared with 39·81 Å for authentic stearic acid. Francis & Piper (1939) give a value of 39·75 Å for the C modification of *n*-octadecanoic acid.

The infrared spectrum of the hydrogenated material was identical with that of authentic stearic acid.

Alkali isomerization. The absorption spectrum of the octadecatetraenoic acid, isomerized under nitrogen for 15 min. at 180° in a 21% (w/w) solution of KOH in glycol (Herb & Riemenschneider, 1952), is shown in Fig. 3.

Oxidation products. The conditions of oxidation and the chromatographic methods used were the same as those described by Whitcutt (1957). The ozonolysis product, when chromatographed, showed the presence of malonic and adipic acid, and traces of glutaric acid.

A further sample was oxidized with CrO_3 and the only volatile products obtained were acetic and propionic acid.

Synthesis and degradation of nonadecatetraenoic acid. The octadecatetraenoic acid (45 mg.) was used to prepare the methyl ester of its higher homologue (see Whitcutt & Sutton, 1956). The ozonized product was treated as described previously, and the resulting dicarboxylic acids were chromatographed on paper. Malonic and pimelic acid, together with traces of adipic acid, were detected.

DISCUSSION

Fig. 2 shows that the resolution achieved by reversed-phase partition chromatography of the acid concentrate was imperfect. As octadecatetraenoic acid is a minor component of pilchard oil and represented only about one-third of the acid concentrate, it was necessary to compromise between purity and the size of samples collected (40–50 mg. from each chromatogram) in order to obtain enough relatively pure material for further investigation. Consequently, the isolated octadecatetraenoic acid contained some impurities situated both on the left (hexadecatrienoic acid) and on the right (eicosapentaenoic acid) side of the chromatographic curve. The presence of these impurities was reflected in chain-length analysis, melting point and X-ray long spacing of the hydrogenated product, as well as in the shape of the ultraviolet curve obtained after alkali isomerization of the unsaturated acid. The amount of each impurity tended to vary, depending on where the fraction was cut. The analytical values showed, however, that the total amount of impurities was not greater than 10%. The purity of the isolated acid was therefore about 90%.

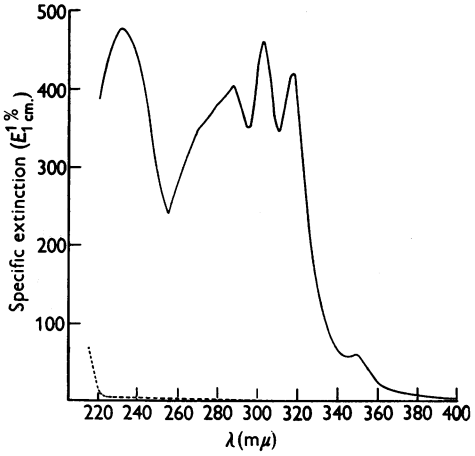

Fig. 3. Ultraviolet absorption of octadecatetraenoic acid before (broken line) and after (continuous line) alkali isomerization. For details, see text.

The iodine value was the only analytical result appreciably different from the theoretical value calculated for octadecatetraenoic acid. Similar acids often give low iodine values, and hexadecatrienoic acid, which was present as an impurity, might have had some influence. Nevertheless, the difference is too small to be accounted for by the presence of acetylenic or allenic unsaturation.

The spectral evidence excluded the possibility of conjugated, *trans*-, terminal or α–β unsaturation (see Whitcutt & Sutton, 1956).

When the acid was alkali-isomerized, conjugated diene, triene and tetraene, together with small amounts of pentaene, were formed. This indicated the presence of $\cdot CH{:}CH \cdot CH_2 \cdot CH{:}CH \cdot$ groups (see de Surville, Sutton & Rivett, 1957). The small amount of conjugated pentaene formed is almost certainly due to the presence of eicosapentaenoic acid, but the presence of traces of a C_{18} pentaenoic acid cannot be excluded. Since this acid would have to be unsaturated in the 3 position, and since such acids are not found normally in nature (see Paschke & Wheeler, 1954), this possibility is improbable.

The oxidation products of octadecatetraenoic acid were acetic, propionic, malonic, adipic and glutaric acid, the last being present in traces. After production of the homologue and subsequent ozonolysis of the resulting nonadecatetraenoic acid, malonic, pimelic and traces of adipic acid were

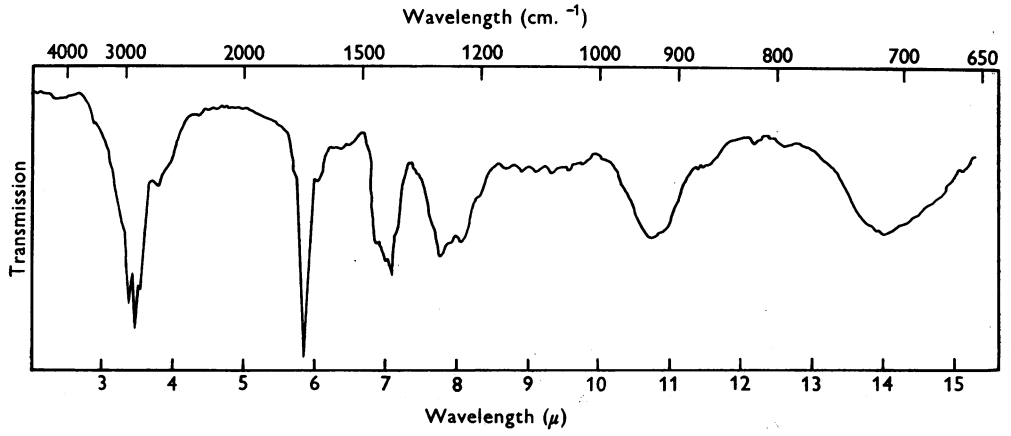

Fig. 4. Infrared absorption of octadecatetraenoic acid. For details, see text.

detected. In both cases malonic acid was by far the most abundant product. The traces of glutaric and adipic acid in the oxidation products of the C_{18} and C_{19} acid respectively, can be explained by the presence of C_{20} and C_{21} pentaenoic acid, from which the above dicarboxylic acids are the known oxidation products. However, the possible presence of small amounts of an isomeric octadecatetraenoic acid cannot be excluded.

The main component of the isolated material therefore has the structure:

$$CH_3 \cdot CH_2 \cdot [CH{:}CH \cdot CH_2]_4 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CO_2H$$

(all-*cis*-*n*-octadeca-6:9:12:15-tetraenoic acid).

So far all the other highly unsaturated acids isolated from pilchard oil which have been reported in this series, as well as all the polyethenoid acids of marine and mammalian origin isolated by Klenk and his co-workers (see Klenk, 1957), exhibit methylene-interrupted conjugation.

SUMMARY

1. *cis*-*n*-Octadeca-6:9:12:15-tetraenoic acid has been isolated from South African pilchard oil, and some of its properties have been determined.

The author thanks Dr W. S. Rapson for his interest in this work, and Drs D. A. Sutton and J. R. Nunn and Mr J. M. Whitcutt for valuable discussions. Microanalyses were done by Miss P. M. Hughes, infrared spectra by Miss W. Albrecht and X-ray long spacings by Dr F. H. Herbstein. The melting point of the octadecatetraenoic acid was determined by Dr S. C. Mossop. This paper is published with the permission of the South African Council for Scientific and Industrial Research.

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Changes in the Distribution of Phosphorus in Platelet-Rich Plasma During Clotting

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In the course of work which has suggested that adenosine triphosphate is concerned in the accumulation of biologically active amines by platelets (Born, Ingram & Stacey, 1956; Born & Hornykiewicz, 1957; Born & Gillson, 1957) it was observed that, when platelet-rich plasma clots, adenosine triphosphate rapidly disappears from the platelets. This paper reports these experiments and others made to determine what happens during clotting to the phosphate groups of adenosine triphosphate and to other phosphate compounds which are present in plasma and in platelets. Some of the results have been published in preliminary form (Born, 1956*a*, *b*, 1957).

METHODS

All glassware used in the isolation of rabbit and human platelets was coated with silicone MS 1107 (Hopkin and Williams Ltd.). When pig platelets were used this was not always done, because pig platelets have little tendency to clump even when in contact with glass.

Blood samples. Pig blood was obtained in the slaughter house. Immediately after the pig was electrically stunned, the throat was slit and venous blood was collected in a polythene container immersed in ice. Rabbit blood was obtained by cardiac puncture. Human blood was obtained by venepuncture. Blood samples were mixed with sodium citrate (final concn. 13 mM) to prevent clotting.

Platelets were isolated at 0–2°. Blood was centrifuged at 500*g* for 15–20 min. and supernatant plasma was